ether. The combined ether extract was washed with brine and then water. The ether solution was then extracted with 3 × 20 mL of 2 N HCl. The acidic aqueous extracts were combined and reduced to dryness several times from absolute EtOH. The residue was recrystallized from EtOH–ether to yield 0.818 g (79%) of white crystals: mp 259–260 °C; NMR (free base, $CDCl_3$, 470 MHz) δ 6.58 (s, 1, Ar H), 6.50 (s, 1, Ar H), 3.81 (s, 3, $OCH_3$), 3.81 (s, 3, $OCH_3$), 2.82 (d of t, 1, CH), 2.73 (d of t, 1, CH), 2.68 (d, 1, CH), 2.54 (d, 1, CH), 1.69 (d of d, 1, CH), 1.65 (d of d, 1, CH), 1.58 (br s, 2, $NH_2$), 1.18 (s, 3, $CH_3$). Anal. ($C_{13}H_{20}ClNO_2$) C, H, N.

**2-Amino-2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene Hydrobromide (3).** The hydrochloride salt **9** (103 mg, 0.4 mmol) was added to 5 mL of 48% HBr and heated at reflux for 1 h under $N_2$. The reaction was concentrated to dryness by rotary evaporation under reduced pressure. The solid was dissolved in 10 mL EtOH and again concentrated to dryness. This process was repeated two more times to remove traces of water. The resulting tan solid was recrystallized from EtOH–EtOAc and dried under high vacuum to yield 80.0 mg (73%) as off-white crystals: mp 248–250 °C; NMR ($Me_2SO$-$d_6$) δ 8.20 (br, 5 H, OH, $NH_3^+$), 6.48 (s, 1 H, Ar H), 6.44 (s, 1 H, Ar H), 2.69 (br s, 4 H, Ar $CH_2$), 1.81 (t, 2 H, $CH_2$), 1.25 (s, 3 H, $CH_3$). Anal. ($C_{11}H_{16}BrNO_2$) C, H, N.

**Conformational Analysis.** $^1H$–$^1H$ vicinal coupling constants were determined between nonaromatic protons of the free bases of the *O,O*-dimethyl ethers **10** and **9**. Samples were run in $CDCl_3$. Chemical shifts are reported for high-resolution spectra relative to the $CHCl_3$ resonance at 7.25 ppm. Decoupling experiments were used to initially determine coupling constants. Subsequent refinements were carried out with use of the modification of LAOCN3[10] implemented in the Nicolet ITRCAL program.[11] Final chemical shifts and coupling constants were checked by generation of spectral simulations and comparison with experimentally obtained spectra.

**Pharmacology.** Following the method of McNay and Goldberg,[12] male mongrel dogs (20–25 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv). A tracheotomy was performed, and respiration was maintained with room air via a Harvard respirator.

The right renal artery was exposed by using a flank incision and retroperitoneal dissection. An electromagnetic flow probe was placed on the artery for measurement of blood flow, and a 25-gauge needle, bent at an 80° angle, was proximally inserted into the artery for drug administration. After infusion of phenoxybenzamine, 5 mg/kg ia, a dose–response curve to dopamine (12, 48, and 190 nmol) was obtained. Increasing doses of the test agonist were administered, up to 3000 nmol (0.82 mg of **3**). All dosages were injected in a fixed volume of 0.2 mL.

For measurement of femoral artery vasodilation and vasoconstriction, sciatic and femoral nerves were left intact and the paw circulation was occluded in all experiments. Initially, dose–response curves of dipropyl DA were generated in each experiment by injecting ia 4-fold increasing doses ranging from 3 to 190 nmol. Test compounds were injected to a maximum of 3000 nmol. Compounds causing vasodilation were also studied after administration of propranolol and domperidone. Propranolol was administered ia in a dose of 2.5 mg/kg and β adrenergic blockade was verified by elimination of isoproterenol-induced vasodilation. Domperidone was administered in a dose of 5 μg/kg iv and $DA_2$ receptor blockade verified by antagonism of DPDA-induced vasodilation.

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# $N^2$-(4-Substituted-2,6-dichlorophenyl)-$N^1$,$N^1$-dimethylformamidines as Antihypertensive and Diuretic Agents[1]

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The synthesis of a series of $N^2$-[4-[(arylmethyl)amino]-2,6-dichlorophenyl]-$N^1$,$N^1$-dimethylformamidines that caused marked blood pressure lowering and/or diuresis in spontaneously hypertensive rats (SHR) is reported. Diuretic activity was not always associated with acute antihypertensive activity. Central nervous system effects, most notably sedation, were observed. Compound **9d**, which lowered arterial blood pressure 37 mmHg in SHR when dosed at 100 mg/kg, was further evaluated in chronic hypertensive dogs because of apparent minimal CNS effects. A reduction in arterial blood pressure of 32 mmHg at 1.0 mg/kg during a 6-h postdosing interval was observed. This response was unrelated to α- or β-adrenergic blockade, angiotensin I antagonism, or neuronal or ganglionic blockade. CNS effects were also observed.

Hypertension, a state of elevated arterial blood pressure, has been determined to be a contributing factor in the development of cardiovascular disease.[2] As a result, the desirability of controlling hypertension has led to the development of antihypertensive drugs that lower blood pressure via a number of mechanisms, including those that act on the central nervous system.

The clinical success of clonidine (1, Catapres)[3] and the subsequent extensive structure–activity relationship studies that resulted[4] led to the development of structurally related drugs represented by guanachlor (**2**)[5] and guanabenz (**3**),[5] which are centrally acting antihypertensive agents.

In this report we describe the results of an investigation on a related series of *N*-[4-[(arylmethyl)amino]-2,6-dichlorophenyl]formamidines **4** synthesized in an attempt to produce a compound that controls blood pressure

(1) These compounds are the subjects of U.S. Patents 4 360 466, 1982; 4 368 335, 1983.
(2) Smirk, F. H. In "Medicinal Chemistry. A Series of Monographs"; Schlittler, E., Ed.; Academic Press: New York, 1967; Vol. 7, p 7.
(3) Hoefke, W.; Kobinger, W. *Arzneim.-Forsch* **1966**, *16*, *1038*.
(4) Timmermans, P. B. M. W. M.; Von Zwieten, P. A. *J. Med. Chem.* **1977**, *20*, 1636 and references therein. A more recent report has shown 2,3,6-substitution may be more effective: Timmermans, P. B. M. W. M.; Jonge, A.; Van Zwieten, P. A.; de Boer, J. J. J.; Speckamp, N. W. *Ibid.* **1982**, *25*, *1122*.
(5) Hoefke, W. In "Antihypertensive Agents"; Engelhardt, E. L.; Ed.; American Chemical Society: Washington, D.C., 1976; p 68.

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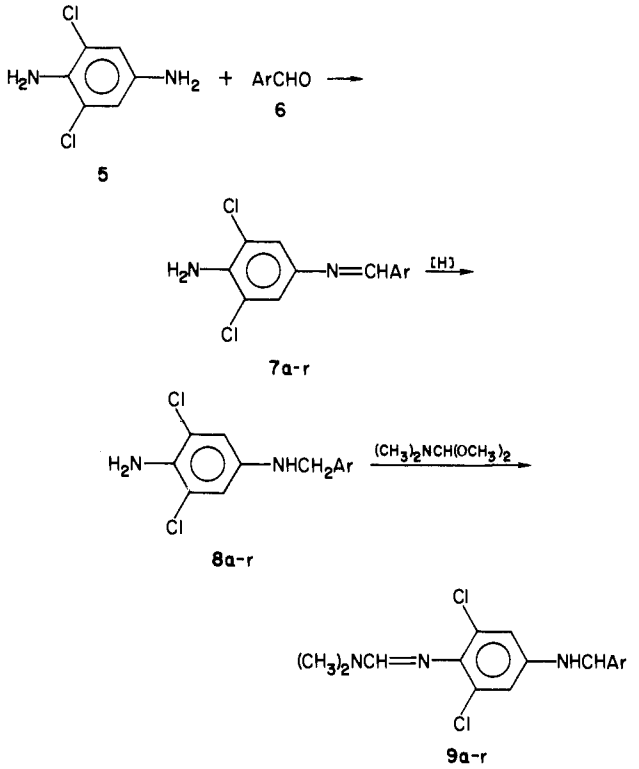
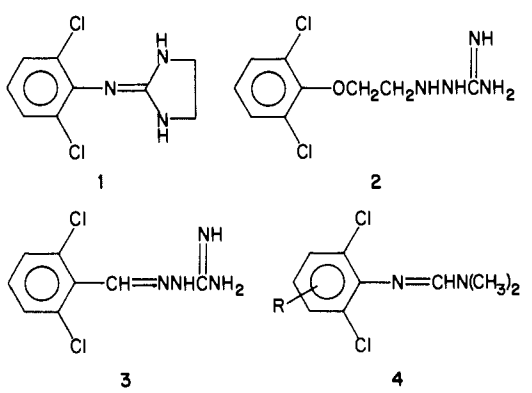
**Scheme I**



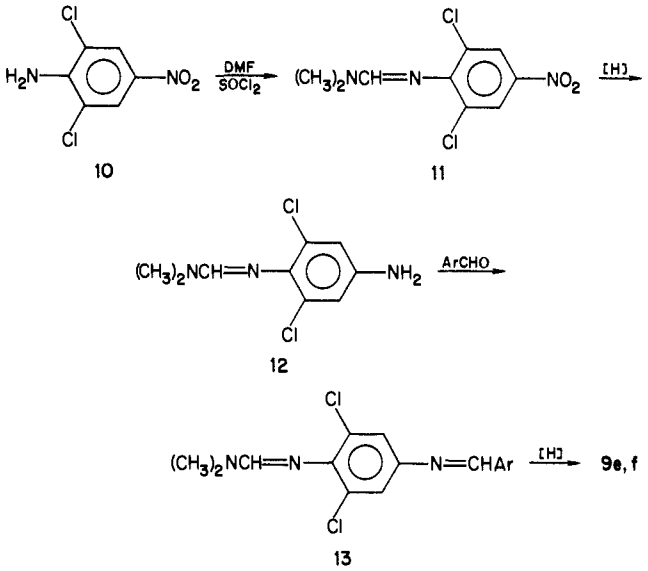
without the undesired sedative properties of centrally acting antihypertensive agents such as **1**, **2**, and **3**.



**Chemistry.** $N^2$-[4-[(Arylmethyl)amino]-2,6-dichlorophenyl]-$N^1$,$N^1$-dimethylformamidines were prepared by either of two routes. In the preferred method (Scheme I), 2,6-dichloro-1,4-phenylenediamine (**5**) was reacted with an equivalent of aldehyde **6** to give a high yield of substituted benzylideneamino derivative **7**, generally obtained analytically pure from the reaction medium. Catalytic hydrogenation or, more conveniently, sodium borohydride reduction of imine **7** gave diamine **8**, which afforded **9** after reaction with dimethylformamide dimethyl acetal. The more reactive lithium borohydride was required to accomplish reduction of **7d** to **8d** and was arbitrarily utilized for the reduction of **7e**, **7g**, **7l**, and **7m** to insure complete reduction.

The structure of **7**, predicted as the product formed by condensation of aldehyde **6** with the least hindered amino group, was established by the unequivocal synthesis of **9e** by an alternative synthetic route (Scheme II). Reaction of 2,6-dichloro-4-nitroaniline (**10**) with thionyl chloride in DMF at 60 °C for 2 h gave formamidine **11**, which was reduced with stannous chloride and hydrochloric acid to **12** in 50% overall yield. Condensation of **12** with an equivalent of appropriate aldehyde **6** in refluxing ethanol

**Scheme II**



gave imines **13e–h** in varying yields depending on the aldehyde used. Aniline **12**, which is relatively electron poor compared to **5**, condensed with aldehyde **6** to form a benzylideneamine at a slower rate and less completely than diamine **5**. Thus, heating **12** with 3-(trifluoromethyl)-benzaldehyde for 3 h in ethanol gave an oil that required chromatographic purification before **13** h could be obtained in crystalline form (53% yield).

Reduction of benzylideneamine **13** was slower than for **7** and gave mixtures that required further purification. Thus, catalytic hydrogenation of **13g**, which required 30–60 min for completion vs. 5 min for **7**, gave mixtures containing the desired product as well as hydrogenolysis products from the benzylamine.

Sodium borohydride reduction of **13e** required 18 h of refluxing in ethanol, while reduction of **13g** with lithium borohydride produced reaction mixtures that included products resulting from the loss of dimethylamine (determined by the absence of the $\delta$ 3.1 $N$,$N$-dimethyl absorption peak in the $^1$H NMR spectrum.

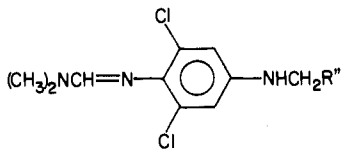
## Results and Discussion

The effects of title compounds **9a–r** on mean arterial blood pressure, heart rate, urine volume, and excretion of sodium and potassium are summarized in Table I. Selected members provided meaningful blood pressure lowering, especially **9d**, **9e**, **9o**, and **9r**, which contain electron-rich 2,4-disubstituted phenyl groups, and **9g** and **9j**, which represent 3-halo monosubstitutions.

Mean blood pressure for these members obtained 4 h after the second daily dose fell 33–40 mmHg relative to the control value of 153 ± 1 mmHg. These compounds also exhibited bradycardia corresponding to reductions in heart rate of 88–168 bpm from the control value of 438 ± 3 bpm. Furthermore, they elicited diuretic and natriuretic effects comparable to clonidine[3] but, as opposed to clonidine, did not selectively foster excretion of sodium relative to potassium.

Although diuresis and natriuresis were associated with many of the analogues, these factors per se did not correlate to blood pressure reduction (i.e., **9h**, **9m**, **9q**). On the other hand, marked blood pressure reduction to levels ≤120 mmHg was always associated with some degree of diuresis (≥16.8 mL/5 h) and natriuresis (≥1.15 mequiv/5 h). Marked blood pressure lowering (≤120 mmHg) was always accompanied by bradycardia (heart rate < 360 bpm). However, bradycardia failed to strictly correlate

**Table I.** Antihypertensive and Diuretic Activity of $N^2$-(4-Substituted-2,6-dichlorophenyl)-$N^1$,$N^1$-dimethylformamidines




| compd | antihypertensive activity R″ | dose, mg/kg | $n^b$ | MABP,[c] mmHg | HR,[d] bpm | diuretic activity[e] vol, mL | $Na^+$, mequiv | $K^+$, mequiv |
|---|---|---|---|---|---|---|---|---|
| control[a] | | | 145 | 153 ± 1 | 438 ± 3 | 4.1 ± 0.2 | 0.39 ± 0.02 | 0.46 ± 0.01 |
| clonidine | | 100 | 5 | 102 ± 5 | 180 ± 15 | 16.4 ± 1.2 | 1.50 ± 0.10 | 0.16 ± 0.04 |
| | | 25 | 4 | 113 ± 3 | 220 ± 16 | NA[f] | | |
| hydrochlorothiazide | | 100 | 5 | 153 ± 5 | 436 ± 13 | 13.2 ± 1.0 | 1.63 ± 0.07 | 0.65 ± 0.05 |
| **9a** | 3,4-$(OCH_3)_2C_6H_3$ | 100 | 2 | 133 | 410 | 8.3 | 0.47 | 0.37 |
| **9b** | 3,4,5-$(OCH_3)_3C_6H_2$ | 100 | 1 | 138 | 360 | 17.5 | 1.40 | 0.57 |
| **9c** | 4-$CH_3CONHC_6H_4$ | 100 | 2 | 150 | 340 | 9.5 | 0.66 | 0.62 |
| **9d** | 2-Cl-4-$N(CH_3)_2C_6H_3$ | 100 | 2 | 116 | 310 | 22.3 | 1.89 | 0.47 |
| **9e** | 3-F-4-$OCH_3C_6H_3$ | 100 | 2 | 113 | 320 | 16.8 | 1.15 | 0.43 |
| **9f** | 2-$OHC_6H_4$ | 100 | 2 | 150 | 340 | 12.3 | 0.72 | 0.68 |
| **9g** | 3-$FC_6H_4$ | 100 | 2 | 120 | 290 | 25.0 | 1.93 | 0.59 |
| **9h** | 3-$CF_3C_6H_4$ | 100 | 2 | 165 | 420 | 21.3 | 2.04 | 0.50 |
| **9i** | 4-$(C_6H_5)C_6H_4$ | 100 | 2 | 143 | 400 | 7.3 | 0.46 | 0.50 |
| **9j** | 3-$BrC_6H_4$ | 100 | 2 | 120 | 350 | 19.3 | 1.90 | 0.48 |
| **9k** | 3,5-$Cl_2C_6H_3$ | 25 | 2 | 139 | 290 | 13.8 | 1.34 | 0.42 |
| **9l** | 4-$N(CH_3)_2C_6H_4$ | 25 | 2 | 171 | 450 | 5.5 | 0.55 | 0.39 |
| **9m** | 4-$N(C_2H_5)_2C_6H_4$ | 25 | 2 | 153 | 400 | 21.3 | 2.02 | 0.71 |
| **9n** | 2-$CH_3$-4-$N(CH_3)_2C_6H_3$ | 25 | 2 | 138 | 270 | 25.5 | 2.59 | 0.60 |
| **9o** | 2-Br-4-$N(CH_3)_2C_6H_3$ | 25 | 2 | 115 | 280 | 19.3 | 1.86 | 0.45 |
| **9p** | 3-Br-4-$N(CH_3)_2C_6H_3$ | 25 | 2 | 173 | 420 | 14.0 | 1.18 | 0.77 |
| **9q** | 2-F-4-$N(CH_3)_2C_6H_3$ | 25 | 2 | 155 | 400 | 23.5 | 2.26 | 0.71 |
| **9r** | 2-$OCH_3$-4-$N(CH_3)_2C_6H_3$ | 25 | 2 | 114 | 270 | 22.5 | 2.16 | 0.75 |
| | | 10 | 3 | 132 ± 1 | 450 ± 13 | 14.8 ± 1.0 | 1.29 ± 0.08 | 0.48 ± 0.04 |

[a] 3% starch. [b] $n$ = number of SH rats (standard error of the mean is provided for $n > 2$). [c] MABP = mean arterial blood pressure. [d] HR = mean heart rate in beats per minute rounded to the nearest 10. [e] Mean = 5 h excretion rate. [f] NA = not available.

with either blood pressure reduction or renal responsiveness (i.e., **9c**).

The presence of a strong hypnotic/sedative effect was widespread within the series and readily observed during testing for antihypertensive and diuretic activities in the SHR screen. Compound **9d**, selected for apparent minimal hypnotic/sedative effect, was further evaluated for antihypertensive efficacy in conscious chronic phase, two kidney–one clip Goldblatt renal hypertensive dogs and for autonomic effector (sympatholytic, antiangiotensin II) blockade in conscious rats.

Results of antihypertensive effect studies in the dog are detailed in Table II. Blood pressure reduction and bradycardia were observed at all doses tested, and duration of both effects increased with dose. The magnitude of the effects of compound **9d** on MABP and HR were comparable to those observed with clonidine. Maximal falls in MABP and HR during a 6-h postdosing interval with 0.5 mg/kg clonidine ($n$ = 4) were 20 ± 5 mmHg and 52 ± 3 bpm, respectively. Maximum mean reductions in MABP and HR with 1.0 mg/kg of compound **9d** ($n$ = 2) were 32 mmHg and 50 bpm, respectively.

Compound **9d** was studied further in conscious SHR in an attempt to elucidate the antihypertensive mechanism of action. An oral dose of 25 mg/kg lowered MABP an average of 38 mmHg within 15 min, an effect that was sustainable for 2 h. The degree to which compound **9d** might block angiotensin II or $\alpha$- and $\beta$-adrenergic receptors, might inhibit norepinephrine release or block ganglionic transmission was assessed by comparing MABP responses to iv injections of angiotensin II, norepinephrine, epinephrine, isoproterenol, tyramine, and dimethylphenylpiperazinium (DMPP) and to postural tilt before dosing with and during the hypotensive response to compound **9d**. Results for this study are presented in Table III.

It is clear that compound **9d** does not block angiotensin II or $\alpha$-adrenergic receptors since pressor responses to angiotensin II, norepinephrine, and epinephrine were unaffected. The potential for $\beta$-adrenergic receptor blockade was not clearly negated. Vasodepressor responses to epinephrine and to isoproterenol were slightly but significantly attenuated. However, it can be reasonably argued that these results are misleading and that compound **9d** is not truly a $\beta$-adrenolytic. First, attenuated vasopressor responses were obtained when MABP was already substantially depressed by compound **9d**, a state in which MABP reduction per se becomes increasingly more difficult to demonstrate. Second, in our hands, single oral doses of propranolol, a $\beta$-adrenolytic of reasonable potency, only marginally lowered MABP in SHR.

Compound **9d** also did not depress neuronal function. MABP elevation to postural tilt and tyramine were unaffected. That the pressor response to DMPP was significantly attenuated is of dubious import in view of the small degree of attenuation, that corresponding starch

**Table II.** Antihypertensive Profile of **9d** in Renal Hypertensive Dogs

| dose, mg/kg | time, h | $n^a$ | MABP,[b] mmHg | HR,[b] bpm |
|---|---|---|---|---|
| 1.0 | 0 | 2 | 140 ± 15 | 110 ± 10 |
| | 1 | | 108 ± 4 | 60 ± 4 |
| | 3 | | 114 ± 9 | 47 ± 3 |
| | 5 | | 128 ± 2 | 66 ± 3 |
| 0.3 | 0 | 3 | 148 ± 3 | 94 ± 22 |
| | 1 | | 111 ± 3 | 60 ± 10 |
| | 3 | | 128 ± 10 | 63 ± 13 |
| | 5 | | 132 ± 10 | 60 ± 20 |
| 0.2 | 0 | 2 | 152 ± 3 | 79 ± 5 |
| | 1 | | 123 ± 1 | 56 ± 8 |
| | 3 | | 129 ± 6 | 58 ± 3 |
| | 5 | | 147 ± 1 | 65 ± 2 |
| 0.1 | 0 | 3 | 146 ± 10 | 120 ± 14 |
| | 1 | | 126 ± 7 | 100 ± 5 |
| | 3 | | 135 ± 5 | 96 ± 3 |
| | 5 | | 144 ± 1 | 113 ± 4 |

[a] Number of dogs. [b] Mean response ± SEM.

Table III. Effect of Compound 9d (25 mg/kg) on Vasopressor and Vasodepressor Responses to Angiotensin II, Postural Tilt, and Autonomic Agonists in SHR

| | starch control ($n$ = 6) | | | compd 9d ($n$ = 6) | | |
|---|---|---|---|---|---|---|
| provocation ($\mu$g/kg) | predose[a] | postdose[a] | $P<$[b] | predose[a] | postdose[a] | $P<$[b] |
| angiotensin II (0.02) | 57 ± 2 | 55 ± 3 | NS[c] | 57 ± 4 | 63 ± 4 | NS |
| norepinephrine (1.0) | 56 ± 4 | 54 ± 4 | NS | 59 ± 3 | 57 ± 3 | NS |
| epinephrine (1.0) | 24 ± 4 | 29 ± 4 | NS | 17 ± 3 | 23 ± 3 | NS |
| epinephrine (2.0) | 39 ± 3 | 45 ± 3 | NS | 42 ± 4 | 50 ± 4 | NS |
| epinephrine (1.0) | −10 ± 2 | −14 ± 2 | NS | −13 ± 2 | −4 ± 2 | NS |
| epinephrine (2.0) | −18 ± 2 | −18 ± 2 | NS | −16 ± 2 | −4 ± 1 | <0.01 |
| isoproterenol (1.0) | −50 ± 5 | −56 ± 6 | NS | −51 ± 2 | −44 ± 2 | <0.05 |
| 75° tilt | 15 ± 5 | 17 ± 3 | NS | 14 ± 2 | 19 ± 2 | NS |
| tyramine (250) | 38 ± 5 | 35 ± 6 | NS | 32 ± 2 | 58 ± 3 | <0.001 |
| DMPP (25) | 62 ± 6 | 46 ± 9 | NS | 58 ± 4 | 33 ± 4 | <0.01 |

[a] MABP, mean ± SEM. [b] Paired Student's $t$ test. [c] NS = not significant.

control rats exhibited attenuation and that responses to postural tilt were unaltered. With these mechanisms of antihypertensive action ($\alpha$- and $\beta$-adrenergic blockades, angiotensin II antagonism, neuronal and/or ganglionic blockade) eliminated from consideration, the possibility that compound **9d** might act through a mechanism analogous to clonidine was enhanced. However, neither this possibility nor the possibility that **9d** itself might relax vascular tissue was tested directly.

## Experimental Section

**Synthetic Methods.** Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Varian HA-100 spectrometer using tetramethylsilane as an internal standard; typical $^1$H NMR shifts are reported. All compounds showed appropriate NMR spectra. Analytical results were within ±0.4% of the theoretical values unless otherwise indicated. Commercially available aldehydes were used without further purification.

**Substituted (Dimethylamino)benzaldehydes.** Aldehydes not commercially available were synthesized according to the method of Campaigne and Archer.[7] The reactions were carried out at 80 °C for 2 h.

From 30 g (0.15 mol) of 3-bromo-*N,N*-dimethylaniline and 150 mL of Vilsmeier reagent, prepared from 16 mL (0.17 mol) of phosphorous oxychloride, there was obtained 20 g (57%), mp 86–88 °C (lit.[8] mp 81–82 °C), of 2-bromo-4-(dimethylamino)-benzaldehyde as colorless needles from heptane.

From 28 g (0.20 mol) of 3-fluoro-*N,N*-dimethylaniline there was obtained 12 g (38%), mp 61–62 °C (lit.[9] mp 62.9–64.5 °C), of 2-fluoro-4-(dimethylamino)benzaldehyde as yellow needles.

From 34 g (0.23 mol) of *N,N*-dimethyl-*m*-anisidine was obtained 10 g (25%) of 1-(dimethylamino)-2-methoxybenzaldehyde, mp 60–62 °C, as pale yellow crystals after distillation, bp 150–152 °C (0.1 mmHg).

Although 2,6-dichlorophenylenediamine is commercially available, it was usually prepared in high yield (75%) and purity by the reduction of 2,6-dichloro-4-nitroaniline with stannous chloride and hydrochloric acid.

**Reduction Method A.** A solution of approximately 20 mmol of **7** in 100 mL of THF containing 500 mg of platinum oxide was reduced in a Paar apparatus with hydrogen at 40 psi for 15 min. The catalyst was removed by filtration and the filtrate evaporated in vacuo. The residue was crystallized from an appropriate solvent.

**Reduction Method B and C.** Approximately 10 mmol of **7** was dissolved in THF or EtOH and stirred with 1 g of lithium borohydride (method B) or sodium borohydride (method C) at ambient temperature for 18 h. Insolubles were removed by filtration and the filtrate evaporated to dryness. Water was added and the product extracted into chloroform. After drying ($MgSO_4$) the solvent was removed in vacuo and the product crystallized from the appropriate solvent.

**2,6-Dichloro-$N^1$-(3-fluoro-4-methoxybenzylidene)-*p*-phenylenediamine (7e).** A solution of 7.1 g (40 mmol) of 2,6-dichloro-*p*-phenylenediamine (**5**) and 6.2 g (40 mmol) of 3-fluoro-4-methoxybenzaldehyde in 50 mL of EtOH was refluxed for 1.5 h. After cooling, the product was collected and washed with a mixture of ether and hexane: yield 8.5 g (68%) of pale yellow crystals, mp 109–110 °C. See Table IV for other compounds prepared by this method. $^1$H NMR ($CDCl_3$) $\delta$ 8.33 (s, 1 H, N=CH), 7.19 (s, 2 H, C-3 and C-4 H), 7.00 (dd, 1 H, $J_{5',6'}$ = 8.0 Hz, $J_{5,F}$ = 8.0 Hz, C-5′ H), 7.52 and 7.70 (dd, overlapping, 2 H, C-2′ and C-6′), 4.43 (s, br, 2 H, $NH_2$), 3.90 (s, 3 H, $OCH_3$).

**2,6-Dichloro-$N^1$-(3-fluoro-4-methoxybenzyl)-*p*-phenylenediamine (8e). Reduction Method B.** A solution of 5.0 g (16 mmol) of **7e** in 50 mL of THF was stirred with 1 g of lithium borohydride for 7 h at ambient temperature. The solids were removed by filtration, and the THF was removed in vacuo. The syrupy residue was stirred in a mixture of water and chloroform for 18 h and separated, the chloroform layer dried ($MgSO_4$) and evaporated, and the residue crystallized from an ether–hexane mixture: yield 3.8 g (75%) yellow crystals, mp 98–99 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.33 (s, 1 H, N=CH), 7.05 (m, 3 H, aromatic), 6.60 (s, 2 H, aromatic), 4.19 (s, 2 H, benzylic $CH_2$), 3.93 (s, 3 H, $OCH_3$).

**$N^2$-[2,6-Dichloro-4-[(3-fluoro-4-methoxybenzyl)amino]-phenyl]-$N^1$,$N^1$-dimethylformamidine (9e). From 8e.** A solution of 3.0 g (9.5 mmol) of **8e** in 20 mL of dimethylformamide dimethyl acetal was refluxed for 7 h and evaporated to a syrup, which was crystallized from a mixture of ether and hexane: yield 1.2 g (34%) of colorless crystals, mp 88–89 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (s, 1 H, formamide CH), 7.00 (m, 3 H, aromatic), 6.60 (s, 2 H, aromatic), 4.18 (s, br, 2 H, benzylic $CH_2$), 3.90 (s, 3 H, $OCH_3$), 3.03 (s, 6 H, $N(CH_3)_2$).

**From 13e.** A 1.0-g (2.7 mmol) sample of **13e** was reduced by method A: yield 670 mg (67%), mp 89–90 °C.

**2,6-Dichloro-4-(nitrophenyl)-$N^1$,$N^1$-dimethylformamidine (11).** A chilled solution of 125 g (0.6 mol) of 2,6-dichloro-4-nitroaniline in dimethylformamide was reacted with 60 mL (0.8 mol) of thionyl chloride dropwise over a period of 30 min while the reaction temperature was allowed to rise to 35 °C. The reaction was warmed to 60 °C for 2 h and cooled and the product precipitated by the addition of 1 L of acetone. The product was collected and washed with acetone: 155 g (87%) colorless needles, mp 119–123 °C.

**$N^2$-(4-Amino-2,6-dichlorophenyl)-$N^1$,$N^1$-dimethylformamidine (12).** A solution of 540 g (2.4 mol) of stannous chloride in 458 mL of 12 N HCl was cooled in an ice–salt bath, and with stirring, 155 g (0.52 mol) of **11** was added in portions such as to maintain a reaction temperature of 75 °C. After stirring at ambient temperature 18 h, the reaction was filtered and the unwashed, damp cake was added to 150 g of ice–$H_2O$. This slurry was neutralized with 10 N NaOH and extracted with chloroform. The product obtained by evaporation of the dried extract ($MgSO_4$) needed no further purification: yield 102 g (84%) of yellow crystals, mp 125–126 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.30 (s, 1 H, formamide CH), 6.60 (s, 2 H, aromatic), 3.54 (s, br, 2 H, $NH_2$), 3.00 (s, 6 H, $N(CH_3)_2$).

**$N^2$-[2,6-Dichloro-4-[(3-fluoro-4-methoxybenzylidene)-amino]phenyl]-$N^1$,$N^1$-dimethylformamidine (13e).** A solution

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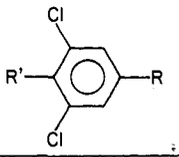
**Table IV.** 1,4-Disubstituted-2,6-dichlorophenyl Derivatives




| compd | R | R′ | yield, % | mp, °C | solvent | formula |
|---|---|---|---|---|---|---|
| **7a** | N=CH-3,5-$(OCH_3)_2C_6H_3$ | $NH_2$ | 88 | 133–135 | | $C_{15}H_{14}Cl_2N_2O_2$ |
| **7b** | N=CH-3,4,5-$(OCH_3)_3C_6H_2$ | $NH_2$ | 84 | 148–150 | | $C_{16}H_{16}Cl_2N_2O_3$ |
| **7c** | N=CH-4-$CH_3CONHC_6H_4$ | $NH_2$ | 69 | 154–156 | | $C_{15}H_{13}Cl_2N_3O$ |
| **7d** | N=CH-2-Cl-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 94 | 139–140 | | $C_{15}H_{14}Cl_3N_3$ |
| **7e** | N=CH-3-F-4-$OCH_3C_6H_3$ | $NH_2$ | 68 | 109–110 | | $C_{14}H_{11}Cl_2FN_2O$ |
| **7g** | N=CH-3-$FC_6H_4$ | $NH_2$ | 69 | 102–103 | | $C_{13}H_9Cl_2FN_2$ |
| **7h** | N=CH-3-$CF_3C_6H_4$ | $NH_2$ | 69 | 124–125 | | $C_{14}H_9Cl_2F_3N_2$ |
| **7i** | N=CH-4-$(C_6H_5)C_6H_4$ | $NH_2$ | 100 | 108–110 | | $C_{19}H_{14}Cl_2N_2$ |
| **7j** | N=CH-3-$BrC_6H_4$ | $NH_2$ | 91 | 136–138 | | $C_{13}H_9BrCl_2N_2$ |
| **7k** | N=CH-3,5-$Cl_2C_6H_3$ | $NH_2$ | 98 | 194–195 | | $C_{13}H_8Cl_4N_2$ |
| **7l** | N=CH-4-$N(CH_3)_2C_6H_4$ | $NH_2$ | 81 | 127–128 | EtOH | $C_{15}H_{15}Cl_2N_3$ |
| **7m** | N=CH-4-$N(C_2H_5)_2C_6H_4$ | $NH_2$ | 47 | 55–57 | EtOH/hexane | $C_{17}H_{19}Cl_2N_3 \cdot 0.5C_6H_{14}$[a] |
| **7n** | N=CH-2-$CH_3$-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 62 | 92–93 | EtOH/hexane | $C_{16}H_{17}Cl_2N_3$ |
| **7o** | N=CH-2-Br-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 90 | 152–153 | | $C_{15}H_{14}BrCl_2N_3$[b] |
| **7p** | N=CH-3-Br-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 81 | 138–140 | | $C_{15}H_{14}BrCl_2N_3$ |
| **7q** | N=CH-2-F-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 75 | 127–128 | | $C_{15}H_{14}Cl_2FN_3$ |
| **7r** | N=CH-2-$OCH_3$-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 68 | 137–139 | | $C_{16}H_{17}Cl_2N_3O$ |
| **8a** | $NHCH_2$-3,5-$(OCH_3)_2C_6H_3$ | $NH_2$ | 65[c] | 84–85 | $CH_3OH$ | $C_{15}H_{16}Cl_2N_2O_2$ |
| **8b** | $NHCH_2$-3,4,5-$(OCH_3)_3C_6H_2$ | $NH_2$ | 46[c] | 96–97 | $CH_3OH$ | $C_{16}H_{18}Cl_2N_2O_3$ |
| **8c** | $NHCH_2$-4-$CH_3CONHC_6H_4$ | $NH_2$ | 71[c] | 125–127 | $CH_3OH$ | $C_{15}H_{15}Cl_2N_3O \cdot 0.5H_2O$ |
| **8d** | $NHCH_2$-2-Cl-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 81[d] | 91–94 | $Et_2O$–hexane | $C_{15}H_{16}Cl_3N_3$ |
| **8e** | $NHCH_2$-3-F-4-$OCH_3C_6H_3$ | $NH_2$ | 75[d] | 98–99 | $Et_2O$–hexane | $C_{14}H_{13}Cl_2FN_2O$ |
| **8g** | $NHCH_2$-3-$FC_6H_4$ | $NH_2$ | 22[d] | 60–61 | EtOAc–hexane | $C_{13}H_{11}Cl_2FN_2$[e] |
| **8h** | $NHCH_2$-3-$CF_3C_6H_4$ | $NH_2$ | 85[c] | >210 dec | EtOH | $C_{14}H_{11}Cl_2F_3N_2 \cdot HCl$ |
| **8i** | $NHCH_2$-4-$(C_6H_5)C_6H_4$ | $NH_2$ | 69[c] | 110–111 | EtOH–hexane | $C_{19}H_{16}Cl_2N_2$ |
| **8j** | $NHCH_2$-3-$BrC_6H_4$ | $NH_2$ | 100[c] | 192–195 dec | EtOH | $C_{13}H_{11}Cl_2BrN_2 \cdot HCl \cdot 0.25H_2O$[f] |
| **8k** | $NHCH_2$-3,5-$Cl_2C_6H_3$ | $NH_2$ | 34[c] | 133–135 | heptane | $C_{13}H_{10}Cl_4N_2$ |
| **8l** | $NHCH_2$-4-$N(CH_3)_2C_6H_4$ | $NH_2$ | 60[d] | 82–83 | EtOH | $C_{15}H_{17}Cl_2N_3$ |
| **8m** | $NHCH_2$-4-$N(C_2H_5)_2C_6H_4$ | $NH_2$ | 18[d] | >120 dec | EtOH | $C_{17}H_{21}Cl_2N_3 \cdot 0.75H_2O$ |
| **8n** | $NHCH_2$-2-$CH_3$-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 81[g] | 108–109 | EtOH | $C_{16}H_{19}Cl_2N_3$ |
| **8o** | $NHCH_2$-2-Br-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 32[g] | 133–135 | EtOH | $C_{15}H_{16}BrCl_2N_3$ |
| **8p** | $NHCH_2$-3-Br-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 62[g] | 87–89 | EtOH | $C_{15}H_{16}BrCl_2N_3$ |
| **8q** | $NHCH_2$-2-F-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 78[g] | 114–115 | $Et_2O$–hexane | $C_{15}H_{16}Cl_2FN_3$ |
| **8r** | $NHCH_2$-2-$OCH_3$-4-$N(CH_3)_2C_6H_3$ | $NH_2$ | 72[g] | 111–113 | | $C_{16}H_{19}Cl_2N_3O$ |
| **9a** | $NHCH_2$-3,4-$(OCH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 64 | 124–125 | $Et_2O$–hexane | $C_{18}H_{21}Cl_2N_3O_2$ |
| **9b** | $NHCH_2$-3,4,5-$(OCH_3)_3C_6H_2$ | $(CH_3)_2NCH{=}N$ | 100 | 137–138 | $CH_3OH$–$H_2O$ | $C_{19}H_{23}Cl_2N_3O_3$ |
| **9c** | $NHCH_2$-4-$CH_3CONHC_6H_4$ | $(CH_3)_2NCH{=}N$ | 71 | 211–213 | EtOH | $C_{18}H_{20}Cl_2N_4O$ |
| **9d** | $NHCH_2$-2-Cl-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 54 | 177–178 | EtOH | $C_{18}H_{21}Cl_3N_4$ |
| **9e** | $NHCH_2$-3-F-4-$OCH_3C_6H_3$ | $(CH_3)_2NCH{=}N$ | 34[h] | 88–89 | EtOH–$Et_2O$ | $C_{17}H_{18}Cl_2FN_3O$ |
| **9f** | $NHCH_2$-2-$OHC_6H_4$ | $(CH_3)_2NCH{=}N$ | 55 | 238–239 | EtOH | $C_{16}H_{17}Cl_2N_3O \cdot HCl \cdot 5H_2O$ |
| **9g** | $NHCH_2$-3-$FC_6H_4$ | $(CH_3)_2NCH{=}N$ | 77 | 239–241 dec | EtOH | $C_{16}H_{16}Cl_2FN_3 \cdot HCl$ |
| **9h** | $NHCH_2$-3-$CF_3C_6H_4$ | $(CH_3)_2NCH{=}N$ | 97 | 214–217 dec | EtOH | $C_{17}H_{16}Cl_2F_3N_3 \cdot HCl$ |
| **9i** | $NHCH_2$-4-$(C_6H_5)C_6H_4$ | $(CH_3)_2NCH{=}N$ | 55 | 165–168 | | $C_{22}H_{21}Cl_2N_3$ |
| **9j** | $NHCH_2$-2-$BrC_6H_4$ | $(CH_3)_2NCH{=}N$ | 89 | 212–219 dec | EtOH | $C_{16}H_{16}BrCl_2N_3 \cdot HCl \cdot H_2O$ |
| **9k** | $NHCH_2$-3,5-$Cl_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 42 | 240–242 dec | EtOH | $C_{16}H_{15}Cl_4N_3 \cdot HCl \cdot 0.25H_2O$ |
| **9l** | $NHCH_2$-4-$N(CH_3)_2C_6H_4$ | $(CH_3)_2NCH{=}N$ | 75 | 79–80 | EtOH | $C_{18}H_{22}Cl_2N_4 \cdot EtOH$[i] |
| **9m** | $NHCH_2$-4-$N(C_2H_5)_2C_6H_4$ | $(CH_3)_2NCH{=}N$ | 63 | 98–99 | EtOH–hexane | $C_{20}H_{28}Cl_2N_4 \cdot 0.25H_2O$ |
| **9n** | $NHCH_2$-2-$CH_3$-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 69 | 166–168 | EtOH–hexane | $C_{19}H_{24}Cl_2N_4$ |
| **9o** | $NHCH_2$-2-Br-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 78 | 135–137 | EtOH–hexane | $C_{18}H_{21}BrCl_2N_4$ |
| **9p** | $NHCH_2$-3-Br-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 50 | 109–110 | EtOH–hexane–$Et_2O$ | $C_{18}H_{21}BrCl_2N_4$ |
| **9q** | $NHCH_2$-2-F-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 54 | 92–94 | EtOH–hexane–$Et_2O$ | $C_{18}H_{21}Cl_2FN_4$ |
| **9r** | $NHCH_2$-2-$OCH_3$-4-$N(CH_3)_2C_6H_3$ | $(CH_3)_2NCH{=}N$ | 49 | 160–162 | EtOH | $C_{19}H_{24}Cl_2N_4O$ |
| **13e** | N=CH-3-F-4-$OCH_3C_6H_3$ | $(CH_3)_2NCH{=}N$ | 75 | 139–141 | | $C_{17}H_{16}Cl_2FN_3O$ |
| **13f** | N=CH-2-$OHC_6H_4$ | $(CH_3)_2NCH{=}N$ | 90 | 113–115 | EtOH | $C_{16}H_{15}Cl_2N_3O$ |
| **13g** | N=CH-3-$FC_6H_4$ | $(CH_3)_2NCH{=}N$ | 60 | 95–96 | heptane | $C_{16}H_{14}Cl_2FN_3$ |
| **13h** | N=CH-3-$CF_3C_6H_4$ | $(CH_3)_2NCH{=}N$ | 53 | 62–64 | hexane | $C_{17}H_{14}Cl_2F_3N_3$ |

[a] $C_6H_{14}$ = *n*-hexane. [b] C, H, N, Br; Cl: calcd, 18.32; found, 17.53. [c] Reduction method A. [d] Reduction method B. [e] C, H, Cl, F; C: calcd, 54.75; found, 54.18. [f] C, H, N, Br; Cl: calcd, 27.48; found, 28.55. [g] Reduction method C. [h] From 8e. [i] Confirmed by NMR.

of 7.0 g (30 mmol) of **12** and 4.6 g (30 mmol) of 3-fluoro-4-methoxybenzaldehyde in 50 mL of MeOH was refluxed 18 h. The product was collected by filtration and washed with ether: yield 8.2 g (75%) of yellow crystals, mp 139–141 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 8.33 (s, 1 H, benzylidene CH), 7.69 (d, 1 H, aromatic), 7.53 (d, 1 H, aromatic), 7.42 (s, 2 H, formamide CH), 7.20 (d, 2 H, aromatic), 7.02 (2 d, 1 H, aromatic), 3.95 (s, 3 H, $OCH_3$), 3.07 (d, 6 H, $N(CH_3)_2$).

**Pharmacological Testing Methods.** The previously reported procedures of Chan et al.[6] were employed to detect acute antihypertensive and diuretic activities and required one to three rats per compound to reach a decision. As the size of the test population increased, the stringency for achieved posttreatment blood pressure and natriuresis lessened. These criteria are tabulated below:

| no. of rats tested | antihypertensive act.: MABP achieved, mmHg | diuretic act.: sodium excreted, mequiv/5 h |
|---|---|---|
| 1 | <117 | >1.21 |
| 2 | <123 | >1.16 |
| 3 | <129 | >1.09 |

This test used 16-week-old male SH rats (Okamoto strain, Taconic Farms) dosed orally by gavage with 100 mg/kg (unless otherwise specified) of test compound dispersed in a starch suspension (3% in normal saline) in a dose volume of 2 mL/kg. Rats were then given an oral load of normal saline (25 mg/kg) and placed in individual metabolism cages, and 0–5-h urine output was collected. Urinary sodium and potassium concentrations were determined by flame photometry. Twenty-four hours after the first dose, rats were redosed with the exception that the 25 mL/kg normal saline load was omitted. Mean arterial blood pressure (MABP) and heart rate (HR) were obtained via direct femoral arterial puncture under local anesthetic 4 h after this second dose.

Additional studies were performed with compound **9d** in a standing colony of chronic phase, two kidney–one clip Goldblatt renal hypertensive dogs. Control MABP and HR were obtained by transdermal femoral arterial puncture and then the compound was given orally in a gelatin capsule in amounts sufficient to deliver 0.1, 0.2, 0.3, and 1.0 mg/kg based on the daily body weight. To insure accurate dosage at the three lowest treatment levels, the compound was extended with lactose to permit weighing of a greater mass. Arterial puncture was repeated at 1-, 3-, and 5-h intervals after dosing to ascertain drug effects. All animals were habituated to the test procedure through their long history of testing.

To investigate the mechanism of antihypertensive action, conscious restrained SHR were used where the caudal artery and vein were catheterized for blood pressure measurement and access for iv injection of test substances. Autonomic agonists were injected at 15-min intervals or longer, if blood pressure did not return to control, and maximum blood pressure changes recorded. Compound **9d** was administered orally by gavage in a 3% starch suspension at a dose of 25 mg/kg and the series of injections repeated after blood pressure had fallen. Pre- and posttreatment responses were compared for significant differences by paired Student's *t* tests. Starch-treated rats were also tested by the same procedure as a negative control.

**Registry No.** **5**, 609-20-1; **6a**, 7311-34-4; **6b**, 86-81-7; **6c**, 122-85-0; **6d**, 1424-66-4; **6e**, 351-54-2; **6g**, 456-48-4; **6h**, 454-89-7; **6i**, 3218-36-8; **6j**, 3132-99-8; **6k**, 10203-08-4; **6l**, 100-10-7; **6m**, 120-21-8; **6n**, 1199-59-3; **6o**, 55875-47-3; **6p**, 56479-63-1; **6q**, 1524-07-8; **6r**, 84562-48-1; **7a**, 84562-23-2; **7b**, 84562-21-0; **7c**, 92366-56-8; **7d**, 84562-19-6; **7e**, 92366-57-9; **7g**, 85103-57-7; **7h**, 85103-60-2; **7i**, 84562-39-0; **7j**, 84562-44-7; **7k**, 84562-37-8; **7l**, 84562-46-9; **7m**, 84562-29-8; **7n**, 84562-62-9; **7o**, 84562-64-1; **7p**, 84562-58-3; **7q**, 84562-66-3; **7r**, 84562-49-2; **8a**, 92366-58-0; **8b**, 85103-52-2; **8c**, 92366-59-1; **8d**, 85103-54-4; **8e**, 92366-60-4; **8g**, 85103-58-8; **8h**, 92366-61-5; **8i**, 85103-63-5; **8j**, 92366-62-6; **8k**, 85103-67-9; **8l**, 92366-63-7; **8m**, 85103-71-5; **8n**, 85103-73-7; **8o**, 85103-75-9; **8p**, 85103-77-1; **8q**, 85103-79-3; **8r**, 85103-69-1; **9a**, 92366-64-8; **9b**, 85103-51-1; **9c**, 92366-65-9; **9d**, 85103-53-3; **9e**, 85103-55-5; **9f**, 92366-66-0; **9g**, 92366-67-1; **9h**, 92366-68-2; **9i**, 85103-62-4; **9j**, 92366-69-3; **9k**, 92366-70-6; **9l**, 92366-71-7; **9m**, 85103-70-4; **9n**, 85103-72-6; **9o**, 85103-74-8; **9p**, 85103-76-0; **9q**, 85103-78-2; **9r**, 85103-68-0; **10**, 99-30-9; **11**, 2350-60-9; **12**, 84562-31-2; **13e**, 84562-30-1; **13f**, 84562-33-4; **13g**, 84562-32-3; **13h**, 84562-34-5; 3-bromo-*N*,*N*-dimethylaniline, 16518-62-0; 3-fluoro-*N*,*N*-dimethylaniline, 2107-43-9; *N*,*N*-dimethyl-*m*-anisidine, 15799-79-8.

# A Potent Multisubstrate Analogue Inhibitor of Human Thymidylate Synthetase[1]

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The synthesis of an 8-deazafolate analogue of the intermediate in the methylation of 2′-deoxyuridylate is described. Alkylation of diethyl 5,6,7,8-tetrahydro-8-deazafolate with 3′-*O*-acetyl-5-(bromomethyl)-2′-deoxyuridine 5′-[bis(trichlorethyl) phosphate], followed by removal of the trichloroethyl groups with a Zn/Cu couple and mild saponification, gave the target inhibitor *N*-[4-[[[2-amino-3,4,5,6,7,8-hexahydro-4-oxo-5-[(2′-deoxyuridin-5-yl)methyl]-pyrido[3,2-*d*]pyrimidin-6-yl]methyl]amino]benzoyl]-L-glutamic acid 5′-monophosphate. The free nucleoside and the 5′-(methyl phosphate) diester were similarly prepared. Each of these reactions yielded a pair of diastereoisomers about C-6 of the reduced deazafolate in approximately a 1:1 ratio. These diastereoisomeric mixtures were evaluated as inhibitors of thymidylate synthetase derived from human tumor (HeLa) cells. The 5′-monophosphate was a potent inhibitor, competitive with respect to both 2′-deoxyuridylate ($K_i$ = 0.06 μM) and tetrahydrofolate ($K_i$ = 0.25 μM). In contrast, the nucleoside and the nucleotide methyl ester were poorer inhibitors by more than 3 orders of magnitude, attesting to the importance of the anionic function at the nucleoside 5′-position in the affinity of an inhibitor for the enzyme active site.

The concept of "thymineless death" (loss of cell viability associated with cessation of DNA synthesis resulting from a lack of thymidylate) was put forth by Cohen[4,5] to explain the effects of thymine deprivation upon *Escherichia coli*. Although this concept has for many years been used to justify thymidylate synthetase (TS) as a target for cancer chemotherapy, only recently has it been clearly demonstrated in mammalian cells.[6]

For two decades, the conventional wisdom has been that the antitumor drug 5-fluorouracil acts via its conversion to 2′-deoxy-5-fluorouridylic acid (FdUMP), which inhibits TS and deprives the cell of thymidylate.[7] That view has been challenged by the demonstration that the ribonucleotide (FUMP) is incorporated into RNA and that the incorporation correlates with cytotoxicity in certain human cells line.[8,9] Furthermore, there are recent demonstrations of low-level incorporation and removal of FdUMP from DNA[10–13] and a new mechanism of action of FUdR was

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